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STEPHEN E. REITER
FOLEY & LARDNER
P.O. BOX 80278
SAN DIEGO, CA 92138-0278

| EXAMINER |
|---|
| WILSON, MICHAEL C |

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**BEFORE THE BOARD OF PATENT APPEALS**

**AND INTERFERENCES**

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Paper No. 30

Application Number: 08/919501

Filing Date: Aug. 28, 1997

Appellant(s): O'Gorman and Wahl

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Teresa Spehar for Stephen E. Reiter

For Appellant

## EXAMINER'S ANSWER

This is in response to the appeal brief filed 3-12-02.

*(1) Real Party in Interest*

A statement identifying the real party in interest is contained in the brief.

*(2) Related Appeals and Interferences*

A statement identifying the related appeals and interferences which will directly affect or be directly affected by or have a bearing on the decision in the pending appeal is contained in the brief.

*(3) Status of Claims*

The statement of the status of the claims contained in the brief is correct.

*(4) Status of Amendments After Final*

The appellant's statement of the status of amendments after final rejection contained in the brief is correct.

*(5) Summary of Invention*

The summary of invention contained in the brief is deficient. The invention involves an ES cell comprising i) a construct encoding recombinase operably linked to a promoter that functions primarily in germ cells. The ES cell is used to make a transgenic animal whose germ cells (e.g. sperm or egg) preferentially express recombinase because of the "germline-specific" promoter. The germ cells expressing recombinase can be used for fertilization of germ cells of the opposite sex comprising DNA encoding a marker protein flanked by recombination sites. Excision of the DNA encoding the marker

protein is intended to occur as a result of recombinase expression in the germ cell during fertilization. Optionally, ES cells comprise both DNA encoding recombinase operably linked to a promoter that functions primarily in germ cells and DNA encoding a marker protein flanked by recombination sites. Excision of the DNA encoding the marker protein is intended to occur after the ES cells becomes an animal during gametogenesis. Recombinase expression in germ cells during gametogenesis causes excision of the DNA encoding the marker protein. The purpose of excising the DNA is to remove a marker gene in an animal or to alter the phenotype of an animal.

***(6) Issues***

The appellant's statement of the issues in the brief is substantially correct. The changes are as follows:

The first issue is whether the claims are enabled as broadly written, specifically, whether the specification enables making and/or using any mammalian ES cells, non-human mammalian ES cells, or animal ES cells comprising DNA encoding recombinase operably linked to "germline-specific promoters" as broadly claimed.

The second issue is whether the claims are definite.

***(7) Grouping of Claims***

The appellant's statement in the brief that certain claims do not stand or fall together is not agreed with.

Claims 12-15, 18-24, 26 and 28-31 stand or fall together because claim 28 is dependent upon claim 26. Claims 12-15, 18, 26 and 32, 34 stand or fall together because claim 32 is dependent upon claim 12. Claims 12-15, 18-24, 26 and 35-39 stand or fall together because claim 35 is dependent upon claim 26.

Claims 12-15, 18-24, 26 and 40-42 stand or fall together because the broad scope of non-human mammalian ES cells (claims 12-15, 18-24, 26) and mammalian ES cells (claims 40-42) are not enabled. Claims 12-15, 18-24, 26 and 43 stand or fall together because claim 43 is dependent upon claim 12. Claims 12-15, 18-24, 26 and 44 stand or fall together because the broad scope of non-human mammalian ES cells (claims 12-15, 18-24, 26) and using pluripotent ES cells to make a non-human animal (claim 44) are not enabled. Claims 12-15, 18-24, 26 and 46 stand or fall together because the broad scope of non-human mammalian ES cells (claims 12-15, 18-24, 26) and rodent ES cells (claim 46) are not enabled. Claims 12-15, 18-24, 26 and 46 stand or fall together because the broad scope of non-human mammalian ES cells (claims 12-15, 18-24, 26) and transgenic rodent made using mammalian ES cells (claim 46) are not enabled. Claims 12-15, 18-24, 26 and 47 stand or fall together because the broad scope of non-human mammalian ES cells (claims 12-15, 18-24, 26) and transgenic mice made using any non-human mammalian ES cells of claim 12 (claim 47) are not enabled. Claims 12-15, 18-24, 26 and 48 stand or fall together because the broad scope of non-human mammalian ES cells (claims 12-15, 18-24, 26) and transgenic mice made using any non-human mammalian ES cells of claim 26 (claim 48) are not enabled. Claims 12-15, 18-24, 26 and 49 stand or fall together because the broad scope of non-human mammalian ES cells (claims 12-15, 18-24, 26) and rodent ES cells (claim 49) are not enabled. Claims 12-15, 18-24, 26 and 51 stand or fall together because the broad scope of non-human mammalian ES cells (claims 12-15, 18-24, 26) and livestock ES cells (claim 51) are not enabled.

Claims 28-31, claims 32, 34, claims 35-39 and claim 43 stand or fall together because they all require non-human mammalian ES cells comprising DNA encoding recombinase operably linked to a germline-specific promoter.

Claims 28-32, 34-39, 43 and claims 40-42 stand or fall together because the broad scope of non-human mammalian ES cells (claims 28-32, 34-39, 43) and mammalian ES cells (claims 40-42) are not enabled. Claims 28-32, 34-39, 43 and claim 44 stand or fall together because the broad scope of non-human mammalian ES cells (claims 28-32, 34-39, 43) and using pluripotent ES cells to make a non-human animal (claim 44) are not enabled. Claims 28-32, 34-39, 43 and claim 46 stand or fall together because the broad scope of non-human mammalian ES cells (claims 28-32, 34-39, 43) and making transgenic rodents using non-human mammalian ES cells (claim 46) are not enabled. Claims 28-32, 34-39, 43 and claims 47, 48 stand or fall together because the broad scope of non-human mammalian ES cells (claims 28-32, 34-39, 43) and transgenic mice made using any non-human mammalian ES cells of claim 12 or 26 (claims 47, 48) are not enabled. Claims 28-32, 34-39, 43 and claim 49 stand or fall together because the broad scope of non-human mammalian ES cells (claims 28-32, 34-39, 43) and using rodent ES cells (claim 49) are not enabled. Claims 28-32, 34-39, 43 and claim 51 stand or fall together because the broad scope of non-human mammalian ES cells (claims 28-32, 34-39, 43) and using livestock ES cells (claim 51) are not enabled.

Claims 40-42 and claim 44 stand or fall together because the broad scope of mammalian ES cells (claims 40-42) and using pluripotent ES cells to make a non-human animal (claim 44) are not enabled. Claim 40-42 and claim 46 stand or fall together because the broad scope of mammalian ES cells (claims 40-42) and using non-human mammalian ES cells to make a rodent (claim 46) are not enabled. Claims 40-42 and claims 47, 48 stand or fall together because the broad scope of mammalian ES cells (claims 40-42) and transgenic mice made using any non-human mammalian ES cells of claim 12 or 26 (claims 47, 48) are not enabled. Claim 40-42 and claim 49 stand or fall together because the broad scope of mammalian ES

cells (claims 40-42) and using rodent ES cells (claim 49) are not enabled. Claim 40-42 and claim 51 stand or fall together because the broad scope of mammalian ES cells (claims 40-42) and using livestock ES cells (claim 51) are not enabled.

Claims 44 and 46 stand or fall together because the broad scope of using pluripotent ES cells to make a non-human animal (claim 44) and using non-human mammalian ES cells to make a rodent (claims 46) are not enabled. Claim 44 and claims 47, 48 stand or fall together because the broad scope of using pluripotent ES cells to make a non-human animal (claim 44) and transgenic mice made using any non-human mammalian ES cells of claim 12 or 26 (claims 47, 48) are not enabled. Claim 44 and 49 stand or fall together because the broad scope of using pluripotent ES cells to make a non-human animal (claim 44) and using rodent ES cells (claim 49) are not enabled. Claim 44 and 51 stand or fall together because the broad scope of using pluripotent ES cells to make a non-human animal (claim 44) and using livestock ES cells (claim 51) are not enabled.

Claim 46 and claims 47, 48 stand or fall together because the broad scope of using non-human mammalian ES cells to make a rodent (claims 46) and transgenic mice made using any non-human mammalian ES cells of claim 12 or 26 (claims 47, 48) are not enabled. Claims 46 and 49 stand or fall together because the broad scope of using non-human mammalian ES cells to make a rodent (claims 46) and using rodent ES cells (claim 49) are not enabled. Claims 46 and 51 stand or fall together because the broad scope of using non-human mammalian ES cells to make a rodent (claims 46) and using livestock ES cells (claim 51) are not enabled.

Claims 47, 48 and claim 49 stand or fall together because the broad scope of transgenic mice made using any non-human mammalian ES cells of claim 12 or 26 (claims 47, 48) and using rodent ES cells

(claim 49) are not enabled. Claims 47, 48 and claim 51 stand or fall together because the broad scope of transgenic mice made using any non-human mammalian ES cells of claim 12 or 26 (claims 47, 48) and using livestock ES cells (claim 51) are not enabled.

Claim 49 and claim 51 stand or fall together because the broad scope of transgenic mice made using rodent ES cells (claim 49) and using livestock ES cells (claim 51) are not enabled.

***(8) Claims Appealed***

The copy of the appealed claims contained in the Appendix to the brief is correct.

***(9) Prior Art of Record***

No prior art is relied upon by the examiner in the rejection of the claims under appeal.

***(10) Grounds of Rejection***

The following ground(s) of rejection are applicable to the appealed claims:

1. Claims 12-15, 18-24, 26, 28-32, 34-44 and 46-51 remain rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a mouse ES cell whose genome comprises a nucleic acid sequence encoding recombinase operatively linked to the MP1 promoter, and a method of making a transgenic mouse comprising implanting said mouse ES cells into a host female such that a transgenic mouse is obtained, wherein spermatid of said transgenic mouse express said recombinase, does not reasonably provide enablement for making and/or using any ES cell comprising DNA encoding recombinase operably linked to any germline-specific promoter as broadly claimed, making any transgenic animal or making any recombinant allele as broadly claimed. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

The specification does not enable making and/or using any non-human mammalian ES cells (claims 12-15, 18-24, 28-32, 34-39, 43 and 46-51), mammalian ES cells (claims 40-42) pluripotental ES cells (claim 44), rodent ES cell (claim 49), or livestock ES cell (claim 51) as broadly required in the claims. The ES cells recited in the claims require DNA encoding recombinase operably linked to a "germline-specific promoter." Therefore, to provide expression in the germline, the ES cells must provide germline transmission of the transgene. Mullins of record (1996, J. Clin. Invest., Vol. 98, pages S37-S40) taught that ES cells providing germline transmission were only available in mice (pg S38, col. 1, para. 1). In particular, the stages of embryo development vary among species. The culture conditions required to maintain cells that provide pluripotency and are capable of germline transmission are dependent upon the species of cells. Thus, the stage at which ES cells are isolated and the conditions required to culture ES cells are unpredictable according to species. The art at the time of filing and the specification does not teach how to make ES cells that provide germline transmission other than mouse ES cells. The specification does not correlate methods of isolating mouse ES cells that provide germline transmission to other methods of isolating other species of ES cells that provide germline transmission. The specification does not teach how to recombine alleles in rodents or mice (claims 35, 46-48) using any non-human mammalian ES cells as broadly claimed. Therefore, the specification does not enable making or using non-human mammalian ES cells, mammalian ES cells, pluripotent ES cells, rodent ES cells or livestock ES cells as broadly written.

The specification does not enable "germline-specific promoters" as claimed. A tissue-specific promoter is described as providing expression "only in T-cells, endothelial cells, smooth muscle cells, and the like" (pg 8, line 14-19). Germline-specific promoters must be encompassed by this definition because

germ cells are a type of tissue. While the specification contemplates using "germline-specific promoters" such as the MP1, (pg 6, lines 1-12), the MP1 promoter does not cause expression only in germ cells. The MP1 promoter also causes expression in ES cells (pg 27, line 29-32). In addition, ProCre/P2Bc male mice demonstrated expression of P2Br, the recombined form of P2Bc, in testes, heart, brain and spleen using PCR (page 24, line 32; page 25, lines 8-13). Therefore, the MP1 promoter is not specific to the germline as claimed because it causes expression in cells other than germ cells. The specification does not provide adequate guidance indicating that the protamine 2, c-kit, ZP1, ZP2 or ZP3 promoters are specific to only germ cells. As such, the specification does not enable any "germline-specific" promoters as claimed.

The transgenics made from the ES cells must express recombinase "specifically" in germ cells to a level capable of excising DNA flanked by recombination sites (para. bridging pg 2-3; pg 12, line 1-5). However, the state of the art at the time of filing was that the phenotype of transgenic mice was unpredictable because of the variability of transgene expression (Mullins of record, pg S37, col. 2, line 7). In addition, Wall of record (1996, Theriogenology, Vol. 45, pages 57-68) taught transgene expression and the physiological result of such expression in livestock was not always accurately predicted in transgenic mice (page 62, line 7). Lewandoski of record taught that germline-specific recombinase activity varied and may not be sufficiently high to mediate excision DNA flanked by recombination sites (1997, Current Biology, Vol. 7, pages 148-151; see page 151, column 1, line 4). Thus, it was unpredictable how to obtain germline-specific recombinase activity that is sufficient to mediate excision of DNA flanked by recombination sites using transgenic animals.

The specification teaches making ProCre transgenic mice by transfecting mouse ES cells with a nucleic acid encoding Cre recombinase operatively linked to the MP1 promoter, a promoter that functions

in sperm (page 19, line 25; page 21, line 3). A construct (P2Bc) comprising i) a loxP-flanked neo gene and ii) β-gal gene, both within the RNA polymerase II gene was used to make ProCre/P2Bc mice (pg 22, lines 1-21), but the specification does not teach whether the mice were made by cross breeding ProCre mice with P2Bc mice or by transfecting ES cells with both a recombinase construct and P2Bc construct. ProCre/P2Bc male mice were bred with wild-type female mice resulting mice carrying a recombined P2Bc gene, namely P2Br, which does not have the neomycin resistance gene (page 22, lines 22-30; note: the specification refers to results in Table 1 on page 22, line 30; however, Table 1 is not present in the instant application).

The specification does not enable one of skill to obtain excision of DNA flanked by recombination sites as claimed using recombinase expressed under the control of a germline specific promoter. The specification does not overcome the unpredictability in the art by providing the parameters required for one of skill in the art to use transgenic animals to obtain germline-specific recombinase activity that is sufficient to mediate excision of DNA flanked by recombination sites. While the specification teaches obtaining excision of DNA flanked by recombination sites in ProCre/P2Bc mice (para bridging pg 22 and 23), the specification does not teach whether the ProCre/P2Bc having sufficient recombinase activity were made by breeding ProCre mice with P2Bc mice, by transfecting ES cells with two constructs or by transfecting ProCre ES cells with the P2Bc construct. Nor does the specification teach sufficient recombinase activity was obtained by obtaining ProCre/P2Bc ES cells which were passaged through gametogenesis as claimed. Given the teachings in the specification taken with the unpredictability in the art, it would have required one of skill undue experimentation to determine how to obtain sufficient recombinase activity that is sufficient to mediate excision of DNA flanked by recombination sites as claimed using transgenic animals.

The specification also teaches transfecting ProCre ES cells with a nucleic acid sequence flanked by two loxP sites (page 25, lines 22-34; page 26, lines 1-7). However, the specification does not teach how to use such ES cells or the phenotype of transgenics made using such ES cells. In fact, the marker gene is excised in the ES cells themselves (indicating the promoter is not specific to the germline as claimed; pg 26, lines 7-26), but the specification does not teach how to use such an ES cell wherein excision of the marker gene occurs within the ES cell itself. Given the unpredictability in the art taken with the guidance provided in the specification, it would require one of skill undue experimentation to determine how to use ES cells having a construct encoding recombinase and a nucleic acid sequence flanked by recombinase sites as claimed.

The specification does not teach how to use an ES cell comprising DNA encoding recombinase operably linked to a germline-specific promoter and DNA encoding recombinase operably linked to a tissue-specific or inducible promoter (claims 31, 36, 39, 40, 41). The specification contemplates using an inducible promoter to facilitate temporal control of recombinase expression in ES cells (page 19, line 10). The specification does not teach why or when to control recombinase expression using tissue-specific or inducible promoters in ES cells that already have germline-specific promoters or why or when two segments would be excised. The specification does not teach the phenotype of transgenic made from such ES cells. Without such guidance it would require one of skill undue experimentation to determine how to use the cells or methods claimed.

Claim 43 is not enabled because the specification does not teach any inactive gene segments which can be used to make a eukaryotic cell of interest by merely introducing the segmenting to an ES cell as claimed. The specification does not teach the steps and inactive gene segments are required to obtain a

biologically active gene or "conditional assembly" of a gene upon passage of the genome through gametogenesis as claimed. Without such guidance, it would require one of skill undue experimentation to determine how to excise a selectable marker such that an inactive gene became active or was "conditionally assembled" as claimed.

Given the teachings in the specification taken with the unpredictability in the art at the time of filing regarding a) how to obtain ES cells that provide germline transmission in species other than mice, b) how to obtain a phenotype of interest in transgenics, c) the lack of correlation in phenotype between transgenic mice and other species, and d) how to obtain adequate gamete-specific recombinase levels capable of excising DNA flanked by recombination sites in transgenics, it would have required one of skill undue experimentation to make and/or use the claims as broadly claimed.

2. Claims 28-32, 34-44 and 46-51 remain rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention for reasons of record.

Claims 28, 32, 35, 40, 43 and 44 remain indefinite because the phrase "passaging the genome derived from said embryonic stem cells through gametogenesis" is unclear. An animal is required for gametogenesis to occur; however, the method does not clearly set forth obtaining an embryo from the ES cells or that the embryo is maintained until gametogenesis occurs. The specification does not describe how a genome is "derived" from an ES cell or how the genome is "passaged" through gametogenesis. It is unclear how "passaging the genome derived from said embryonic stem cells through gametogenesis"

relates to "spermatogenesis" or "oogenesis". It is unclear if the phrase encompasses ES cells in culture as on page 26, lines 1-26 or fertilization as on pg 22, both of which can cause marker excision.

Claim 35 remains indefinite because the phrase "introducing a nucleic acid fragment flanked by at least two recombination target sites into embryonic stem cells of claim 26" remains unclear. It is unclear if the fragment in claim 35 is selectable marker in claim 26 or a second construct.

Claim 43 remains indefinite because the body of the claim and the preamble of the claim are not commensurate in scope and because it is unclear whether the DNA produced in the body of the claim correlates with the "functional gene(s)", "gene(s) of interest" in the preamble, an "activated" form of the "inactivated" gene or whether it is some other DNA present in the cell.

***(11) Response to Argument***

Applicants argue the examiner has not provided reasoning or evidence in support of the rejection (pg 12). Applicants argument is not persuasive. Numerous references establishing the state of the art at the time of filing, reasoning, and the teachings in the specification have been used to support the enablement rejection (see "Grounds of Rejection" above).

Applicants argue the specification enables ES cells of any non-human mammal because mouse ES cells known in the art are a model for other mammals and because Mullins taught pluripotent rat, sheep and cattle ES cells capable of producing chimeric offspring (pg 13). Applicants argument is not persuasive. First, claims 40-42 and 44 are not limited to non-human mammals; claims 40-42 encompass recombining alleles using any mammalian ES cell and claim 44 encompasses using any pluripotent ES cell to make a non-human animal. Second, the claims require "germline-specific" promoters that express recombinase in germline cells. Thus, germline transmission is essential to the invention. Mullins states ES cells had been

isolated from other species, e.g. rat, sheep, and cattle; however, Mullins clearly states that ES cells providing germline transmission were only available in mice (pg S38, col. 1, para. 1). Therefore, rat, sheep and cattle ES cells comprising DNA encoding recombinase operably linked to a "germline-specific" promoter could not be used in the instant invention because the transgene would not be expressed in germline cells. While the specification contemplates other animals, the specification does not provide any guidance regarding how to use ES cells to obtain germline transmission in any animals other than mice. In fact, even to this date, germline transmission of a transgene is not known using ES cells other than mice. The specification does not provide a use for ES cells comprising a transgene having a "germline-specific" promoter that does not express the transgene in the germline. In view of the unpredictability in the art taken with the teachings in the specification, it would have required one of skill undue experimentation to determine how to make and/or use ES cells comprising DNA encoding recombinase operably linked to a "germline-specific" promoter other than mouse ES cells.

Applicants argue the specification enables "germline-specific promoters" because the specification teaches various germline-specific promoters (pg 6, line 1-12) and the promoters listed have equivalent function as MP1 (pg 14-16). Applicants argue the MP1 promoter is "germline-specific" because it is well known in the art that "tissue-specific" provide "substantially exclusive expression" as described on page 8, lines 14-18. Applicants arguments are not persuasive. Applicants provide abstracts from Peschon (1987), Peschon (1989) and Zambrowicz (1993) indicating the MP1 promoter was "spermatid-specific." The claims are not limited to spermatid-specific promoters. In addition, the abstracts do not teach testing in heart, brain or spleen. Thus, it cannot be determined from the abstracts whether the art accepted definition of "germline-specific" promoters allowed for expression in other tissues. More importantly, on page 8, line

18, of the instant application, "tissue-specific" expression is defined as expression "only in T-cells" for example. The MP1 promoter causes expression in spermatid, ES cells, the heart, brain and spleen. As such, the MP1 is not a "germline-specific" promoter because it does not express protein "only" in germline cells. The specification does not provide the expression pattern using any other promoter listed on pg 6. The specification does not provide adequate evidence indicating the promoters listed have the same "specificity" as MP1 or provide adequate recombinase expression in transgenics such that a nucleic acid sequence flanked by recombination sites would be excised. Without such guidance, the specification does not enable "germline-specific promoters" as claimed.

Applicants argue the claims do not require a phenotype of transgenic animals. Applicants go on to argue the invention provides methods of generating an array of mutations in mammals, such as conditional mutations and null mutations. Applicants state the invention provides animals having a "molecular phenotype" for analysis. Therefore, applicants argue the specification need not teach the phenotype of any of the animals produced by the method. Applicants arguments are not persuasive. Methods of recombination to produce animals having an array of mutations without teaching how to use the animals produced are not enabled. Obtaining an animal having a "molecular phenotype" for further analysis is not adequate guidance for one of skill to use the ES cell. Rather, it is a wish to know the biological property of animals made from ES cells having that genotype. Furthermore, the "molecular phenotype" of an animal is in reality the "genotype." For example, the specification teaches ProCre/P2Bc mice have a unique genotype; however, the specification does not teach the phenotype of ProCre/P2Bc mice or how to use ProCre/P2Bc mice. The specification does not teach how to make conditional mutations and null mutations in animals. The specification does not provide adequate guidance for one of skill to predict the

phenotype of such animals. Since the phenotype of transgenic animals was unpredictable at the time of filing (see Mullins and Wall, both of record), taken with the guidance in the specification, applicants do not enable one of skill how to use the ES cells or methods as broadly claimed to make animals having an array of mutations.

Applicants argue Example 3 teaches transfecting ProCre ES cells with a nucleic acid sequence flanked by two loxP-sites to make ProCre/P2Bc mice. Applicants argument is not persuasive. Page 22, lines 10-21, is the description of making ProCre/P2Bc mice.

"To determine whether ProCre nucleic acid construct would efficiently recombine a target allele, males were generated that contained a ProCre nucleic aid construct and a target for Cre-mediated recombination. This "P2Bc" (Pol II, β-GAL, conditional) target (Figure 1) was created using homologous recombination in ES cells to insert a loxP-flanked neomycin cassette and a β-gal coding sequence into the first exon of the locus coding for the large subunit of RNA polymerase II. Cre-mediated recombination of the loxP sites was expected to delete the intercalated sequence, creating "P2Br" allele (Pol II, β-GAL, recombined)."

The description does not teach ProCre ES cells were transfected with the loxP-flanked neomycin cassette. The description does not teach ES cells containing both constructs were used to make ProCre/P2Bc mice. The description does not teach the ES cells in Example 5 having two constructs were used to make ProCre/P2Bc mice. As such, it cannot be determined how to make the ProCre/P2Bc mice.

Applicants argument that ProCre males bred with P2Bc females did not result in recombination because only spermatogenesis would activate the recombinase and create the recombinant P2Br allele (pg 19, 1st full para., of arguments; pg 23, line 17, of specification) is contradictory to Example 5 which

teaches ES cells comprising DNA encoding recombinase transfected with a constructs flanked by loxP sites has recombination without becoming an embryo (and therefore without going through spermatogenesis).

Applicants argue the method claims are enabled as written because they are supported by working examples. The method claims as written are not limited to the parameters required to cause excision of selectable markers, recombination of alleles, e.g. transfecting ProCre ES cells with loxP flanked construct mice, implanting the ES cells into a pseudopregnant female, obtaining offspring that have gone through gametogenesis or obtaining recombination because of gametogenesis. Furthermore, the specification does not enable obtaining recombination by obtaining an animal having gone through gametogenesis because ES cells having two constructs had recombination (Ex 5).

Applicants argue an animal having a marker gene is a tool for generating animals of interest and that the utility of such animals are self-evident and well-established to one of skill in the art. Applicants argument is not persuasive. The methods result in the excision of the marker gene. The art at the time of filing does not provide an enable use for an animal having the marker gene excised. Therefore, it is not readily apparent how to use such an animal. The specification does not provide an enabled use for the animal. Therefore, the specification does not enable using an ES cell that has a marker gene excised or methods of excising a marker gene from an animal.

Applicants argue the phrase "passaging the genome derived from said embryonic stem cells through gametogenesis" is definite. Applicants discuss the crux of the invention (pg 23 of response) but the discussion does not correlate the crux of the invention with the "passaging" step. Applicants state one of skill in the art would recognize the only way to generate recombinase would be to passage the ES cells through gametogenesis. Applicants arguments are not persuasive. The specification does not teach what

the metes and bounds of passaging an ES cell through gametogenesis are. ES cells do not go through gametogenesis; ES cells do not divide thereby directly producing germ cells. Gametogenesis is the development of germ cells within an animal and does not involve ES cells. ES cells may be "passaged" in culture and may be implanted into a uterus to obtain an embryo or offspring. Gametogenesis may occur in the offspring. ES cells are not "passaged" through gametogenesis. The phrase simply does not reflect the method steps required for gametogenesis or to use ES cells to obtain an animal having germ cells. Furthermore, ES cells that do not go through gametogenesis have recombination (Example 5, pg 27, line 23); therefore, the phrase does not reflect the method steps required to obtain recombination.

Applicants argue "introducing a nucleic acid fragment" is clear because it is clearly a second construct and because the fragment in 35 could not be the construct referred to in claim 26. Applicants argument is not persuasive. A selectable marker can be considered a nucleic acid fragment because it is part of the genome of another organism. For example, the β-gal gene is a nucleic acid fragment isolated from bacterial DNA. It cannot be found where the specification contemplates transfecting ES cells with a construct comprising a selectable marker flanked by recombination sites and a construct comprising a nucleic acid fragment flanked by recombination sites. As such the phrase remains indefinite.

Applicants argue the phrase "thereby producing a DNA which encodes a functional gene" is definite. Applicants argue the phrase reflects the phrase "conditional assembly of function genes(s)." Applicants argue one of skill would recognize this DNA is an "assembly of the individual inactive gene segments by recombination into a functional gene" (pg 26 of response). Applicants arguments are not persuasive. The body of the claim does not require the "DNA encoding a functional gene" is assembled or "conditionally" assembled as in the preamble. The body of the claim does not require the inactive gene

becomes active or that the "DNA which encodes a functional gene" is the active form of the inactive gene. As such, it cannot be determined if the DNA is DNA encoding recombinase, DNA having a knockout excised that becomes functional, or DNA within a knockout that becomes functional. The claim does not clearly set forth the result of introducing the inactive gene segments.

For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

Michael C. Wilson

MICHAEL C. WILSON
PATENT EXAMINER

MCW/mcw

May 17, 2002

DEBORAH J. REYNOLDS
SUPERVISORY PATENT EXAMINER
TECHNOLOGY CENTER 1600
conferee

REMY YUCEL, PH.D
SUPERVISORY PATENT EXAMINER
TECHNOLOGY CENTER 1600
Conferee